The yin and the yang of follicular lymphoma cell niches: Role of microenvironment heterogeneity and plasticity.

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Abstract

Follicular lymphoma (FL) results from the malignant transformation of germinal center B cells and is characterized by recurrent genetic alterations providing a direct growth advantage or facilitating interaction with tumor microenvironment. In agreement, accumulating evidences suggest a dynamic bidirectional crosstalk between FL B cells and surrounding non-malignant cells within specialized tumor niches in both invaded lymph nodes and bone marrow. Infiltrating stromal cells, macrophages, and T/NK cell subsets either contribute to anti-tumor immune response, or conversely form a tumor supportive network promoting FL B cell survival, growth, and drug resistance. This review depicts the phenotypic heterogeneity and functional plasticity of the most important FL cell partners and describes their complex interplay. We also unravel how malignant B cells recruit and subvert accessory immune and stromal cells to trigger their polarization towards a supportive phenotype. Based on these observations, innovative therapeutic approaches have been recently proposed, in order to benefit from local anti-tumor immunity and/or to selectively target the protective cell niche.

KEYWORDS: Cell interactions, Stromal cells, follicular helper T cells, macrophages

1. Introduction

Follicular lymphoma (FL) is the most frequent indolent lymphoma and is considered virtually incurable with high response rates to therapy but frequent relapses [1]. Progression to aggressive diffuse large B-cell lymphoma (DLBCL) occurs in about 35% of cases, an event associated with poor outcome [2]. Malignant FL B cells express germinal center (GC) B-cell markers such as BCL6 and CD10, have somatically mutated immunoglobulin variable genes with ongoing intraclonal diversification, and display a gene expression profile of centrocytes, indicating that FL results from the malignant transformation of GC-derived B cells [3]. The genetic hallmark of FL is the t(14;18) translocation associated with an overexpression of the anti-apoptotic protein BCL2, actively repressed in normal GC B cells. However, this founder genetic event is detected at a low frequency in most healthy individuals within peripheral blood IgM memory B cells, the so-called FL-like cells (FLLC) [4], suggesting that additional driver genetic events are required to complete cell transformation. Accordingly, genome-wide profiling has recently shed new lights on the mutational landscape in FL and delineated a hierarchical model of successive genetic events supporting FL tumorigenesis [1, 5].

Besides the failure of primary FL cells to survive and grow autonomously *in vitro*, the major role of the microenvironment in FL development and evolution has been highlighted by several seminal observations. First, like their normal counterpart, malignant FL B cells are found admixed with lymphoid stromal cells, macrophages, and follicular helper $CD4^{pos}$ T cells (T_{FH}) in GC-like follicles within invaded lymph nodes (LN) [6]. In addition, bone marrow (BM) infiltration found in up to 70% of patients at diagnosis is characterized by an ectopic differentiation of lymphoid-like stromal cells [7] and local enrichment in $CD4^{pos}$ T cells [8] suggesting a critical dependence of malignant B cells to this specific supportive cell niche. Despite these similarities, some differences in cell composition and organization exist between LN and BM niches [8, 9]. In agreement, different subclones could be detected within BM and LN, and BM FL cells are characterized by a lower cytological grade and proliferation [9-11]. These data support the hypothesis that trafficking in various specific microenvironments could contribute to FL clonal selection and molecular heterogeneity [12]. Second, several highly frequent genetic alterations are not oncogenic *per se* but favor the crosstalk of FL cells with neighboring cells. Among them, mutations in *TNFRSF14/HVEM* affecting its expression or binding to the

inhibitory receptor BTLA could contribute to the maintenance and supportive activity of $BTLA^{hi}$ FL-infiltrating T_{FH} [13, 14]. Moreover, more than 90% of FL cases display unusual sites for N-linked glycosylation within their immunoglobulin variable regions introduced during the somatic hypermutation process (SHM) [15]. Added glycans contain oligomannoses that might interact with C-type lectins expressed by myeloid cells in the microenvironment, allowing FL cells to receive antigen-independent but cell contact-dependent survival signals through their BCR [16]. Finally, several studies based on expression profiling and immunohistochemistry have proposed a panel of prognostic biomarkers reflecting the number, activation, and/or spatial organization of infiltrating immune cells, further emphasizing the central role of FL microenvironment [17]. In the landmark study performed on whole tumor biopsies, the clinical outcome of FL patients was primarily predicted by molecular features of non-malignant cells and not by specific genetic characteristics of tumor B cells [18]. However, these studies led to highly contradictory results, in part due to treatment heterogeneity, and remained essentially descriptive without transposition of the data into more functional and mechanistic approaches.

Our current knowledge of the relationship between FL B cells and their microenvironment has been hindered by four main technical pitfalls: i) the lack of true FL B-cell lines; ii) the lack of relevant transgenic mouse model of FL; iii) the difficulty to establish FL xenografts in immunocompromised mice in the absence of T-cell help and mature secondary lymphoid organs; iv) the heterogeneity and plasticity of the numerous cell subsets involved in FL cell growth, associated to their limited survival and proliferation *in vitro*. Nevertheless, several recent studies have provided interesting clues illustrating the two faces of FL microenvironment; *i.e.* its capacity to exert anti-tumor activity by itself or by potentiating the efficacy of FL-targeting drugs *versus* its capacity to favor directly and indirectly FL B-cell growth. This review will try to integrate them in a comprehensive view of the intricate FL cell niche. A related interesting question is how malignant B cells co-opt and divert their microenvironment to create a conducive niche in LN and BM and how this niche is modified after treatment and support FL relapse. A better understanding of the ambivalent role of FL microenvironment would be useful to select the more relevant biomarkers for patient stratification and prognosis. It will also make it possible to design new microenvironment-targeted treatments, a field that recently gained increasing attention in B-cell lymphomas.

2. Microenvironment can inhibit FL cell growth

FL has long been considered as particularly immune responsive based on reports of spontaneous regressions, high response rates to monoclonal antibodies (mAb) associated with a long-lasting vaccinal effect, and good biological responses to vaccination using tumor-specific idiotype or immunogenic neoplastic cells [19-22]. Several immune cell subsets could contribute to this anti-tumor activity and provide useful biomarkers and potential therapeutic targets.

2.1 Cytotoxic lymphoid cells

$CD8^{pos}$ T cells are major actors of anti-tumor immunity and an increased $CD8^{pos}$ T-cell infiltrate is correlated to a better FL prognosis [23]. Similarly, high levels of blood $CD3^{pos}$, $CD4^{pos}$, and $CD8^{pos}$ predict favorable outcome in patients treated with rituximab [24]. Using 3-D tissue imaging, Laurent *et al.* described a rich infiltrate of functional $CD8^{pos}$ cells containing granzyme B^{pos} lytic granules in the interfollicular spaces [25]. T cells at the follicular border form lytic synapse-like structures with FL B cells, suggesting a tonic control of malignant cell trafficking and FL progression. However, a global $CD8^{pos}$ T-cell exhaustion as well as dysfunctional synapses with FL B cells have been reported in biopsy specimens [26, 27]. In addition, intratumoral regulatory T cells (Treg) have been shown to inhibit *in vitro* degranulation and cytotoxic activity of infiltrating $CD8^{pos}$ T cells exposed to lymphoma B cells [28].

Beside antigen-driven cytotoxicity of $CD8^{pos}$ T cells, innate anti-tumor cytotoxicity involved essentially NK cells and $\gamma\delta$ T lymphocytes (Figure 1). Our knowledge of *in situ* NK cells in FL is limited, and the low frequency of $CD56^{pos}$ cells on malignant tissue sections has not been associated with the progression of the disease [29]. However, we could hypothesize an induction of NK-DC crosstalk by therapeutic mAb, which could trigger tumor antigen-specific T cell immunity [30]. Considering $\gamma\delta$ T cells, $V\gamma9\delta2$ T cells recognize tumor phosphoantigens, like isopentenyl pyrophosphate (IPP), and are able to kill *in vitro* a wide variety of tumor cell lines, as well as primary FL B cells [31]. Whereas $\gamma\delta$ T cells could migrate into GC within normal secondary lymphoid organs [32], immunohistochemistry studies revealed that these cells display mainly perifollicular localization and are represented at lower density in FL LN tissues, compared to reactive LN [33]. Moreover, those FL B cells retaining HVEM expression could inhibit proliferation of $BTLA^{pos}$ infiltrating $V\gamma9\delta2$ T

cells [34]. In agreement, we found a lower *in vitro* expansion capacity of FL infiltrating Vγ9δ2 T cells in response to a combination of their pharmacological agonist bromohydrin pyrophosphate (BrHPP) with IL-2 (our unpublished data). FL B cells were shown to express ULBP proteins, the ligands for NKG2D activating receptor and an increase in circulating ULBP-responsive Vδ1 T lymphocytes have been described in FL patients [35]. However, the role of $V\delta2^{neg}$ γδ T cells in FL pathogenesis remains poorly understood.

Finally, NK cells, γδ T lymphocytes, and a subset of $CD8^{pos}$ T cells share the capacity to mediate antibody-dependent cellular cytotoxicity (ADCC), an important mechanism of anti-tumor immune response. The association between Rituximab clinical efficiency and a specific polymorphism in CD16/FcγRIIIa resulting in a modulation of affinity for IgG1 revealed the critical role of CD16-expressing cells in the activity of this anti-CD20 mAb [36].

Overall, cytotoxic cells of both innate and adaptative immunity could efficiently kill lymphoma B cells but this antitumor immune response is actively counteracted by tumor escape mechanisms affecting immune cell recruitment and activation.

2.3 Myeloid cells

Tumor-associated macrophages (TAM) exhibit a dual role in FL pathogenesis, as underlined by the opposite predictive value of a high TAM content, depending on treatment schedule. In fact, high numbers of $CD68^{pos}$ or $CD163^{pos}$ TAM are associated with adverse outcome in FL patients treated with conventional chemotherapy, whereas this prognosis value is abrogated or even inversed when Rituximab is combined with chemotherapy [37-39]. These data suggest that FL TAM could favor tumor progression but also contribute to the clinical efficacy of antibody-based anti-lymphoma drugs (Figure 2). Accordingly, B cell depletion with anti-CD20 mAb in mouse models prominently depends on macrophages, and more specifically on their expression of activating FcγR [40]. In addition, for rituximab-mediated tumor clearance in human, antibody-dependent cellular phagocytosis (ADCP) mediated by macrophages probably plays a key role beside that of NK-mediated ADCC [41]. In particular, Rituximab and Ofatumumab show high direct ADCP capacities *in vitro* and elicit TNF-α release by macrophages, which could indirectly contribute to NK cell activation [42]. Interestingly, alternatively activated M2 macrophages were shown to

display *in vitro* a greater phagocytic capacity towards Rituximab-opsonized B cells from chronic lymphocytic leukemia (CLL), when compared to M1 proinflammatory macrophages [43]. This was associated with a differential regulation of FcγR expression by polarizing cytokines. In agreement, several reports demonstrate that IL-4 decreases expression of CD64/FcγRIa whereas IL-10 up-regulates all classes of FcγR and favors CD32a/FcγRIIa-mediated phagocytosis [44]. FL TAM display a $CD16^{neg}CD32a^{hi}CD64^{hi}$ phenotype and CD32a is primarily involved in the phagocytosis of anti-CD20-opsonized primary FL B cells *in vitro* (our unpublished data). Altogether, the overexpression of both IL-4 and IL-10 within FL microenvironment [45] could modulate the TAM phenotype, including expression levels of FcγRs and phagocytic properties in the presence of therapeutic antibodies.

The clinical relevance of ADCP has been further underlined by the demonstration that FL and DLBCL B cells overexpress CD47, a transmembrane protein that enables evasion of phagocytosis through binding to the inhibitory receptor signal regulatory protein (SIRP)-α on macrophages [46]. Accordingly, a blocking mAb targeting CD47 restores phagocytic activity of macrophages *in vitro* and synergizes with Rituximab for the elimination of human lymphoma in xenotransplant models in NOD-*scid* $Il2rg^{null}$ (NSG) mice. It was recently demonstrated that the genetic determinant favoring human cell engraftment in NOD-based immunodeficient mouse models is a *Sirpa* gene polymorphism allowing recognition of human CD47 by mouse phagocytes and inhibiting human grafted cell engulfment [47]. This observation could have potential impact for the development of new xenograft models of FL.

Altogether, TAM appear as a highly plastic cell subset involved in antitumor immunity, in particular through FcγR-related ADCP, a process actively inhibited by CD47-expressing malignant B cells.

2.4 Relevance for the design of new therapies

The demonstration that several immune cell subsets could efficiently trigger FL B-cell death is difficult to reconcile with the general lack of clinical response following classical antigen-specific immunotherapy strategies. In particular, despite promising proof-of-principle studies, the results of phase III randomized trials examining the clinical impact of idiotypic vaccination in FL were disappointing [21]. Since idiotypes have been shown to be immunogenic, these negative results are likely due to

mechanisms of avoiding immune cell recruitment, recognition, and lysis by malignant B cells. A better understanding of the relationships between B cells and their immune microenvironment will be highly useful to design new treatments that could overcome immune escape and enhance clinical efficacy of chemotherapy and antibody-mediated immunotherapy (Figure 3).

Such approaches are currently explored with immunomodulators like lenalidomide that is evaluated in clinical trials in FL, in combination with Rituximab. Lenalidomide has pleiotropic activities including a substantial capacity to activate NK cells, to increase T-cell proliferation and function, and to enhance macrophage-mediated ADCP of Rituximab-coated tumor cells [48]. The main molecular target of lenalidomide is the E3 ubiquitin ligase cereblon that was recently shown to trigger the induction of cytokine production by T cells [49]. Interestingly, FL infiltrating $CD4^{pos}$ and $CD8^{pos}$ T cells display immunological synapse dysfunction with impaired F-actin polymerization [27]. This defect could be reversed after *in vitro* treatment of both FL and autologous T cells with lenalidomide. A recent report pinpoints lenalidomide capacity to rescue LFA-1-dependent T-cell adhesion and motility in CLL patients by restoring Rho GTPase activity [50]. The relevance of this mechanism for the clinical activity of lenalidomide in FL remains to be evaluated.

Another important research field is the engineering of modified antibodies with optimized effector properties [51]. GA101 afucosylated anti-CD20 mAb is currently under clinical evaluation in FL and is supposed to mediate greater NK-cell ADCC through a higher affinity for CD16/FcRγIIIA that abrogates the negative impact of the unfavorable FcRγIIIA polymorphism [42]. However, GA101 recently demonstrated inferior ADCP compared with rituximab suggesting that new Fc modifications are required to improve specific binding to CD32a and enhance phagocytosis [52]. Besides targeting directly tumor cells, a new generation of antibodies has been designed to stimulate immune cells in the microenvironment (reviewed in [53]). Among them, agonistic antibodies targeting the inducible co-stimulatory molecule CD137 could favor ADCC and T-cell activation and enhance the antilymphoma activity of anti-CD20 mAb [54]. Similarly, antibodies blocking KIR, the NK inhibitory receptor family, could be useful despite their systemic activation of resting NK cells [55]. Finally, promising immunological and clinical results have been obtained using combination of rituximab, BrHPP, and IL-2 in relapsed FL patients, suggesting that γδ

T cells could be activated *in vivo* and trigger direct cytotoxic activity as well as ADCC owing to their inducible CD16 expression [56]. Targeting immune cells to increase their effector functions should thus be considered as a promising therapeutic strategy in FL, in particular with the aim of optimizing mAb-driven cytotoxicity.

3. Microenvironment can favor FL cell growth

Besides its potential role in tumor eradication, FL microenvironment revealed individual tumor supportive activity of each cell subset, including stromal cells, T_{FH}, or TAM, on malignant B-cell recruitment, survival, proliferation, and drug resistance. In addition, the FL cell niche should be envisioned as a dynamic network of cell interactions where the various cell compartments also contribute to migration, expansion, activation, and polarization of each other.

3.1 Stromal cells

Cancer-associated fibroblasts (CAF) are phenotypically and functionally different from their normal counterpart and play a key role in tumor development and progression in various cancer models [57]. In FL, LN and BM CAF essentially display some features of lymphoid stromal cells, a heterogeneous cell compartment organized as three specialized cell niches within normal secondary lymphoid organs [58, 59]: i) the mesenchymal stromal network of the T-cell zone is formed by fibroblastic reticular cells (FRC), which provide a foothold for antigen delivery, immune cell recruitment, motility, interaction, and homeostasis through the release of extracellular matrix components, IL-7, VEGF, nitric oxyde, and homeostatic chemokines CCL19, CCL21 and CXCL12; ii) follicular dendritic cells (FDC) drive CXCL13-dependent attraction of B cells and T_{FH} within the GC where they promote the selection of high affinity B cells through the retention and presentation of antigens as immune complexes; iii) finally, marginal reticular cells (MRC) deliver small antigens to cognate B cells though specific follicular conduits. The main common feature of lymphoid stromal cells is to derive from resident local precursors and to require both tumor necrosis factor (TNF)-α and lymphotoxin (LT)-$\alpha1\beta2$ for their maturation and maintenance as immunologically competent cells. In addition, human LN contain *bona fide* mesenchymal stromal cells (MSC) that can be triggered to adipocyte, osteoblast, and chondrocyte lineages but also to FRC differentiation in response to a combination of TNF-α and LT-$\alpha1\beta2$ [60]. However, the exact origin of

human lymphoid stromal cells remains elusive in both normal and FL-invaded LN and BM. Moreover, FL LN stromal cells displayed altered *in situ* phenotype compared to normal LN, including a uniform and marked activation of transglutaminase expressing FRC-like network whereas follicle stromal cells progressively loose classical FDC markers [61-63]. The mechanisms and consequences of these phenotypic modifications are unknown, in particular because functional analysis of native stromal cells remains a highly challenging issue.

MSC obtained after culture of invaded FL BM (FL-MSC) support more efficiently the growth of malignant B cells than MSC obtained from healthy donor BM (HD-MSC) [64]. To better understand this result, we compared the gene expression profile of FL-MSC and HD-MSC and revealed that FL-MSC in the BM are ectopically committed to a FRC-like differentiation. In agreement, FRC-like cells obtained from HD-MSC in response to TNF-α and LT-$\alpha 1\beta 2$ priming are more powerful to drive malignant B-cell survival than HD-MSC themselves [60]. The tumor-promoting capacity of FL-MSC remains to be molecularly understood (Figure 4).

Stromal cells have been involved in the recruitment of malignant FL B cells through the release of CXCL12 and CXCL13 [60, 65]. They also contribute directly and indirectly to B-cell survival and drug resistance. Accordingly, interaction of malignant B cells and FDC was recently suggested to upregulate MDR1, an ABC transporter triggering multidrug resistance in FL B cells compared to DLBCL [66]. Among the paracrine supportive factors produced by stromal cells, Hedgehog (Hh) ligands, B cell-activating factor of the TNF family (BAFF), IL-15, hepatocyte growth factor (HGF), and the adhesion molecule CD106 have all been proposed to contribute to the antiapoptotic effect of stromal cells on normal and malignant GC B cells [67-74]. Paracrine Hh signaling might also favor stroma-mediated chemotolerance in indolent lymphomas by upregulating the drug transporter ATP-binding cassette (ABC)G2 [75], whereas adhesion of VLA-4^{pos} FL B cells to $CD106^{pos}$ stromal cells protects them from rituximab-induced apoptosis [76]. The role of FDC-derived Wnt5a, Notch ligands, and PGE2 in GC-derived lymphomas has not been explored to date. In addition, the question of whether stromal cells transfer paracrine information to malignant B cells remains unanswered. BM-MSC from patients with multiple myeloma release exosomes containing specific microRNA and proteins that support the growth and dissemination of malignant plasma cells [77]. It would be important to

clarify the role of such microvesicles in the crosstalk between stromal cells and B cells in FL.

Finally, beyond the numerous individual factors suspected to trigger stromal protection of neoplastic cells, a recent paper revealed that BM-MSC promote gluthatione synthesis and survival of CLL B cells [78]. Given the central place of oxydative metabolism in cancer development, it is tempting to speculate that such mechanism could also be involved in the supportive activity of stromal cells in FL context.

FL stromal cells are likely organizers of FL cell niche. In particular, FL-MSC overexpressed CCL2 that favors the recruitment of monocytes and triggers their differentiation into proangiogenic and anti-inflammatory TAM-like macrophages [64]. Interestingly, MSC isolated from spontaneous lymphomas in mouse also overexpress CCR2 ligands and recruit more macrophages than BM-MSC [79]. In addition, IFN-γ primes human and mice FRC to produce the immunosuppressive enzymes indoleamine-2,3 dioxygenase (IDO) and nitric oxide synthase 2 (iNOS), respectively [80, 81]. Since IFN-γ is upregulated within FL microenvironment [80], such primed FRC could thereafter inhibit T-cell proliferation and contribute to tumor immune escape.

In conclusion, stromal cells play a central role in FL pathogenesis through both a direct tumor B-cell supportive activity and an indirect effect on the orchestration of FL cell niche.

3.2 $CD4^{pos}$ T cells

Genes related to $CD4^{pos}$ T cells represent a prominent part of the FL specific prognostic signature at diagnosis emphasizing the pivotal role of this subset (Figure 1). Despite a global profile of exhausted T cells associated with a high proportion of PD1 and/or $TIM3^{pos}$ cells [26, 82], FL helper T cells also display a more activated phenotype than reactive LN helper T cells suggesting a more complex phenotype than previously anticipated [83]. In agreement, fully functional PD-1-expressing T_{FH} are enriched within FL LN thus challenging the idea of a classical anergic T-cell-rich tumor niche [45]. T_{FH} were initially identified as $CD4^{pos}$ T cells expressing CXCR5, allowing their localization in follicular areas of secondary lymphoid organs. Recent data broadened the definition of this compartment, and defined it as a distinct helper T-cell lineage, under the control of BCL6, and playing a central role in GC B-cell

localization, selection, and differentiation in normal follicles [84]. Nevertheless, beside this unique functional definition, the T_{FH} compartment is more heterogeneous than previously assumed, and could be subdivided into several subsets, based on the secretion of various cytokines formerly assigned to other helper T cell lineages, *i.e.* IFN-γ, IL-4, or IL-17 shared with Th1, Th2, and Th17 cells; respectively. FL-T_{FH} display a specific gene expression profile compared to tonsil-T_{FH}, with an overexpression of *IL4*, *IL2*, *IFNG*, and *TNF* [85]. High levels of IL-4, essentially produced by $T_{FH,}$ have been associated with a STAT6 and Erk-dependent FL B-cell activation [45, 86] and recent evidences highlighted a potential role of T_{FH}-derived CD40L in malignant B-cell survival [85, 87]. Beside this direct protumoral activity, FL-T_{FH} could also modulate the FL supportive niche through their expression of *TNF* and *LTA* that sustain differentiation and maintenance of the B-cell supportive lymphoid stroma network [60]. In addition, FL-T_{FH} secrete high amounts of IFN-γ, thus inducing the expression by stromal cells of the tryptophan-catabolizing enzyme IDO [80] and could contribute by their overexpression of IL-4 to the polarization of TAM within the malignant cell niche [88].

Analyzes of dissociated FL biopsies revealed also a higher frequency of Treg compared with non-malignant LN or tonsils [85, 89]. Treg have been experimentally described to inhibit the anti-tumor immune responses in FL by suppressing the proliferation and activity of intratumoral $CD4^{pos}$ and $CD8^{pos}$ T cells [28, 89]. Elevated levels of soluble IL-2Rα have been involved in the inhibitory activity of FL-Treg and predict reduced survival in FL [90]. More importantly, an excessive number of $Foxp3^{pos}CXCR5^{hi}$ follicular Treg (T_{FR}) has been specifically reported in FL neoplastic follicles. Strikingly, the follicular localization of $Foxp3^{pos}$ T cells rather than their absolute number was recently associated with a worse overall survival, suggesting that T_{FR} are the more relevant Treg subset for FL biology [91]. These cells share phenotypic characteristics with T_{FH}, and express a higher level of BCL6, the master regulator of T_{FH} differentiation pathway, than classical Treg [85]. T_{FR} also strongly express the co-stimulatory molecule ICOS, as reported for a subset of Treg with strong suppressive functions and able to induce IL-4-secreting T cells [92]. Suppressive T_{FR} have also been described in mice. They control the GC reaction by limiting T_{FH} number and inhibiting the selection of non-cognate B cells [93]. Nevertheless, the targets of the specific FL-T_{FR} subset are currently unknown. It is

tempting to speculate that FL-T_{FR} inhibit anti-tumor responses, thus explaining the worse prognosis associated with their expansion. However, we could not exclude that FL-T_{FR} also contribute to a limitation of the FL-T_{FH} compartment, in agreement with a lower proportion of $KI67^{pos}$ FL-T_{FH}, compared to tonsil-T_{FH} (our unpublished data). If this hypothesis is confirmed, the high proportion of FL-T_{FH} should result from a preferential commitment of activated $CD4^{pos}$ T cells into T_{FH} differentiation pathway, or an increased survival of differentiated T_{FH} in response to tumor-specific surrounding growth factors, rather than a proliferation of a mature T_{FH} compartment.
Altogether, these results highlight the role of various $CD4^{pos}$ T-cell subsets in FL B-cell growth as well as immune escape. In particular, T_{FH} and Treg are strongly modulated both quantitatively and qualitatively during FL pathogenesis.

3.3 Myeloid cells

Although several studies have proposed TAM number as a prognostic marker, knowledge of their direct role in B-cell growth and how they cooperate with neighboring infiltrating T cells and stromal cells is still rudimentary (Figure 2). BAFF is a well-known myeloid-derived B-cell growth factor. Even if BAFF expression is not increased in FL patients [94], a polymorphism in *TNFSF13B/BAFF* was associated with an increased risk of developing FL, whereas a germline mutation in *TNFRSF13C/BAFF-R* was specifically detected in 10% of FL patients in association with a stronger BAFF-induced signaling [95, 96]. Another potential TAM-mediated pathway in FL is BCR signaling. In fact, whereas a recent study suggests that BCR from a subset of FL patients recognize self-antigens potentially retained on the surface of FDC [97], the majority of FL B cells express mannosylated BCR able to interact with C-type lectins DC-SIGN (CD209) and Mannose Receptor (CD206) independently of antigens [15, 16]. CD209 and CD206 are known to be upregulated on M2 macrophages and on TAM in solid tumors [98], a situation mirrored in FL by the overexpression of IL-4 by T_{FH} [45]. FL-T_{FH} also overexpress CD40L that both increases IL-15Rα expression on myeloid cells [99] and confers IL-15 sensitivity to B cells through induction of STAT5 expression and activation [70]. In agreement, FL-TAM overexpress *IL15* compared to tonsil macrophages and FL B cells overexpress *STAT5A* compared to normal GC B cells. Interestingly, IFN-γ, which is also upregulated in FL microenvironment [80], was shown to increase the migration of macrophages in response to CCL2 through an upregulation of STAT1 expression

[100]. Such activation loop should be important for the recruitment of macrophages by $CCL2^{hi}$ FL stromal cells and the presence of $STAT1^{pos}CD68^{pos}$ macrophages in the vicinity of FL B cells was associated with unfavorable outcome [101]. Finally FL-TAM could contribute to local immune escape through the release of immunosuppressive molecules like IL4I1 [102] but also to angiogenesis. Accordingly, increased angiogenic sprouting correlates both with elevated numbers of $CD163^{pos}$ macrophages and poor prognosis [103]. In parallel to the description of *in situ* TAM, circulating $CD14^{pos}$ HLA-DR^{lo} suppressive monocytes have been identified in B-cell NHL [104] and the absolute monocyte count is inversely correlated with overall survival in FL and DLBCL [105]. Overall, myeloid compartment display specific features in FL and contribute to FL pathogenesis through the release of tumor growth factors, proangiogenic molecules, and immunosuppressive mediators.

3.4 Relevance for the design of new therapies

Since interaction of FL B cells with their microenvironment is postulated to be an important mediator of drug resistance and disease relapse, targeting B-cell adhesion and/or retention within tumor niches has recently emerged as a promising therapeutic approach (Figure 3). Anti-VLA-4 mAb natalizumab has been shown to overcome stroma-mediated resistance to Rituximab [76]. In addition, disrupting the CXCL12/CXCR4 axis using the CXCR4 antagonist perixafor (AMD3100) or cell-penetrating peptides targeting CXCR4 may augment the effects of anti-CD20 mAb [106, 107]. Interestingly, the Btk inhibitor PCI-32765, the PI3K inhibitor GS-1101, and Syk inhibitors do not only target BCR signaling but also impair chemokine networks and reduce CLL cell retention in protective microenvironment [108-110]. The combination of these two properties could be highly useful in FL. Lenalidomide also decreases CXCL12 production by stromal cells and alters CLL migration by targeting Rho protein activity [111, 112]. Finally, the tyrosine kinase inhibitor imatinib was demonstrated to impair xenografted lymphoma B-cell growth through a direct targeting of vascular mural cells resulting in loss of tumor vascular integrity [113]. This innovative finding paves the way for the introduction of antiangiogenic agents in our therapeutic arsenal in FL.

Besides stromal cells, $CD4^{pos}$ T-cell help is crucial for FL survival and growth and represents an interesting druggable target. Antagonist mAb to PD-1 and PD-L1 showed both a good safety profile and antitumor activity in some metastatic cancers [114]. They are supposed to block inhibitory PD-1 signaling on tumor infiltrating T

cells or to deplete PD-1^{pos} anergic T cells depending on antibody isotype. In FL, PD-1 is not only expressed on anergic T cells but also on fully functional T_{FH} and T_{FR}, and PD-L1/PD-1 pathway has been recently demonstrated to inhibit proliferation and function of both cell types [115, 116]. The use of anti-PD-1 mAb in this specific context should thus be highly valuable but would also benefit from extensive patient monitoring to unravel the exact target of these drugs. Of course, the recent development of other immune-checkpoint inhibitors, such as anti-CTLA4 mAb should also be considered for patients with FL.

Beyond stimulation of effector immune cells, inhibition of protumoral T and stromal cells through kinase inhibitors or antagonistic mAb emerges as a valuable therapeutic approach in FL.

3. Malignant B cells can subvert their microenvironment

It is now well-established that tumor cells could subvert their molecular and cellular environment to favor their own growth, and to minimize anti-tumor immune response. Accordingly, the recruitment and polarization of the various microenvironment cell subsets in FL is at least partly governed by the malignant clone (Figures 1 & 4).

Indeed, FL B cells directly contribute to the differentiation and maintenance of the dense FRC meshwork within invaded LN [60]. This FRC commitment is partly dependent on TNF-α secretion by malignant B cells, as exemplified for the induction of CCL2 in HD-MSC after contact with primary FL B cells. Invasion of BM by malignant GC B cells expressing TNF-α and LT-$\alpha 1\beta 2$ likely induces ectopic lymphoid-like stromal cells.

Malignant B cells also modify their chemokine environment through a direct secretion of CCL22 that recruits Treg. In addition, they contribute to the polarization of helper T cells. In particular, GC-derived lymphomas skew the balance of Th17 *versus* Treg differentiation in favor of an increased frequency of induced Treg, and both CD27-CD70 and CD28-CD80/CD86 axes are involved in this process [117, 118]. Moreover, induction of TIM-3 on anergic T cells relies on malignant B-cell-derived IL-12 [26] whereas expression of multiple inhibitory ligands on B cells has been proposed to trigger T-cell synapse defect [119]. Despite data suggesting a thymic origin of mice T_{FR} in physiological conditions [93], we could not exclude that the specific FL-T_{FR} subset emerges at least in part from a tumor-driven *in situ* conversion of T_{FH} into induced Treg. In support of this hypothesis, a strong correlation exists between FL-

T_{FH} and FL-T_{FR} contents [85]. Whereas the mechanisms of FL-T_{FH} expansion remain unknown, they should arise from a preferential GC-recruitment and/or commitment of activated helper T cells into T_{FH}. FL B cells could be involved in this process through their overexpression of IL-6 and ICOS ligand (ICOS-L), compared to tonsil GC B cells (our unpublished data). First, it is now well documented that IL-6, in association with IL-21, promotes BCL6 expression in activated $CD4^{pos}$ T cells [120]. Second, ICOS-L expression by follicular bystander B cells has been recently demonstrated to control the recruitment of activated $CD4^{pos}$ T cells into GC allowing their further differentiation into T_{FH} independently of cognate T-B or T-DC interaction [121]. In the context of FL, such co-stimulation independent function of ICOS is presumably important, as suggested by the fully polyclonal repertoire of infiltrating FL-T_{FH} (our unpublished data). Thus, FL B cells are able to trigger recruitment, polarization, and maintenance of $CD4^{pos}$ T cells that in turn promote their survival and growth.

4. Concluding remarks

Genetic and functional studies corroborate the general concept that immune and stromal microenvironment plays a proactive role in the development and progression of FL and determines clinical behavior and response to treatment. The major goal of numerous recent studies was to unravel the different facets of FL cell niche and to define how they interact with each other. However several burning questions remain unsolved. Longitudinal follow-up of microenvironment composition will be helpful to define the kinetic of B cell/microenvironment bidirectional interactions. When do the first modifications of LN and BM niches occur and more specifically could some of them be detected at the FLLC pre-malignant stage? Comparison of microenvironment composition at diagnosis, in the context of residual disease, and in relapse would be instrumental to identify the minimal cell niche protecting malignant B cells from drug-induced cytotoxicity and to better understand the impact of treatments on the various microenvironment cell subsets. The role of the microenvironment in the selection of malignant B-cell subclones should be further analyzed. In particular, what are the molecular features of BM *versus* LN FL cells depending on their specific cell niche? Altogether, developing new tools to better understand microenvironment network may provide innovative tailored strategies to disrupt supportive niches whereas taking advantage of anti-lymphoma infiltrating cells.

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Conflict of interest statement
The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. Dual role of infiltrating T/NK cells in follicular lymphoma cell niche
FL-T_{FH} support malignant B-cell growth through CD40L and IL-4 signaling. Conversely, cytotoxic $CD8^{pos}$ T cells, γδ T cells, and NK cells display anti-tumoral activity and CD16-mediated ADCC is a major mechanism of action of Rituximab mAb. FL B cells subvert antitumor response leading to T-cell immune synapse dysfunction and T-cell exhaustion, to a preferential recruitment of Treg, and to an imbalance in Treg/Th17 polarization in favor of induced Treg. In addition, malignant cells could encourage T_{FH} commitment through their overexpression of ICOS-L and IL-6.

Figure 2. Dual role of infiltrating macrophages in follicular lymphoma cell niche
Tumor-associated macrophages (TAM) contribute directly to malignant B-cell growth through BCR, IL-15, and BAFF signaling, and display proangiogenic and immunosuppressive properties. Conversely, FL-TAM participate to the therapeutic efficacy of anti-CD20 mAb through B-cell phagocytosis, a process inhibited by CD47/SIRPα axis, and activation of cognate cytotoxic T cells. FL-T_{FH} cooperate with FL-TAM for promoting STAT5 activation in B cells and produce cytokines, including IL-4, IL-10, and IFN-γ, involved in TAM migration, polarization, and function.

Figure 3. New drugs to target FL tumor microenvironment
Lenalidomide exerts pleiotropic activities including activation of NK cells, T cells, and macrophage-mediated antibody dependent phagocytosis. Similarly, inhibitors of Btk and Syk exhibit broader mechanisms of action than initially anticipated and target not only BCR-mediated signal but also B cell/stromal cell interactions. Stromal cell niche could also be disrupted using CXCR4 antagonists and anti-VLA4 mAb. Several therapeutic mAb, including anti-PD-1, anti-CTLA4, anti-CD47, and anti-CD137, target tumor immune cells instead of malignant B cells whereas BrHPP is a specific γδT-cell agonist. Finally, imatinib was recently shown to compromise tumor-associated microvasculature through a selective inhibitory effect on pericytes.

Figure 4. Role of infiltrating stromal cells in follicular lymphoma cell niche

Stromal cells recruit and support directly the growth of FL B cells through a combination of chemokines, adhesion molecules, and cytokines. The role of antigen-presentation by FDC remains speculative. This protumoral property is strongly influenced by the local cytokine context. TNF-α (TNF) and LT-$\alpha 1\beta 2$ (LT) produced by malignant B cells and T_{FH} trigger stromal cell engagement into lymphoid stroma differentiation whereas IFN-γ could favor production of T-cell inhibitory enzyme IDO. Finally, FL stromal cells efficiently recruit TAM through the specific release of CCL2 chemokine.

Figure 1

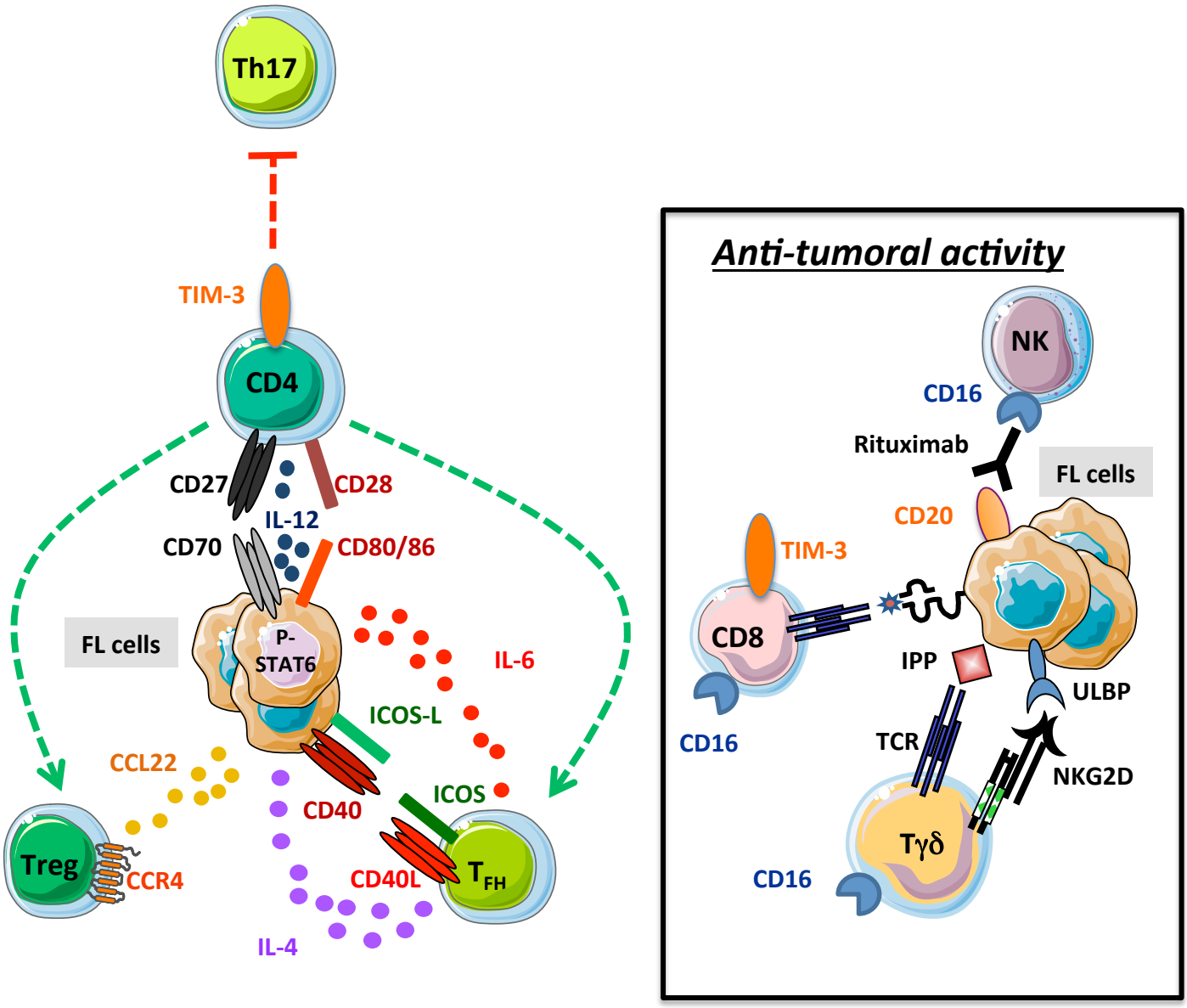

Pro-tumoral activity
Th17
TIM-3
CD4
CD27
CD28
IL-12
CD70
CD80/86
FL cells
P-STAT6
IL-6
ICOS-L
CCL22
ICOS
CD40
Treg
CCR4
CD40L
T FH
IL-4
Anti-tumoral activity
NK
CD16
Rituximab
FL cells
CD20
TIM-3
CD8
IPP
ULBP
CD16
TCR
NKG2D
Tγδ
CD16

Figure 2

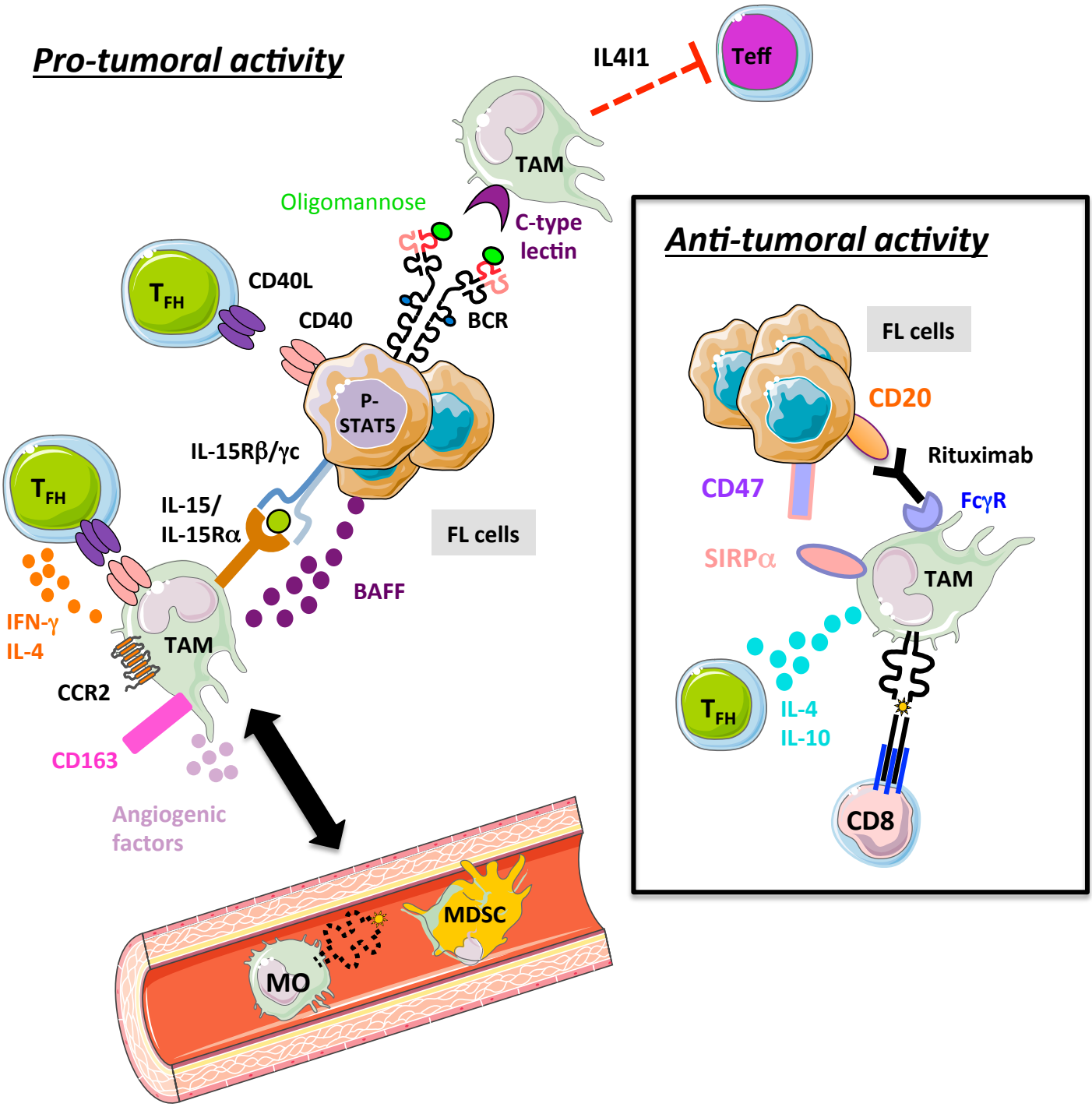

Pro-tumoral activity
IL4I1
Teff
TAM
Oligomannose
C-type lectin
BCR
CD40L
CD40
TFH
P-STAT5
IL-15Rβ/γc
IL-15/ IL-15Rα
FL cells
BAFF
IFN-γ
IL-4
TAM
CCR2
CD163
Angiogenic factors
MDSC
MO
Anti-tumoral activity
FL cells
CD20
Rituximab
CD47
FcγR
SIRPα
TAM
TFH
IL-4
IL-10
CD8

Figure 3

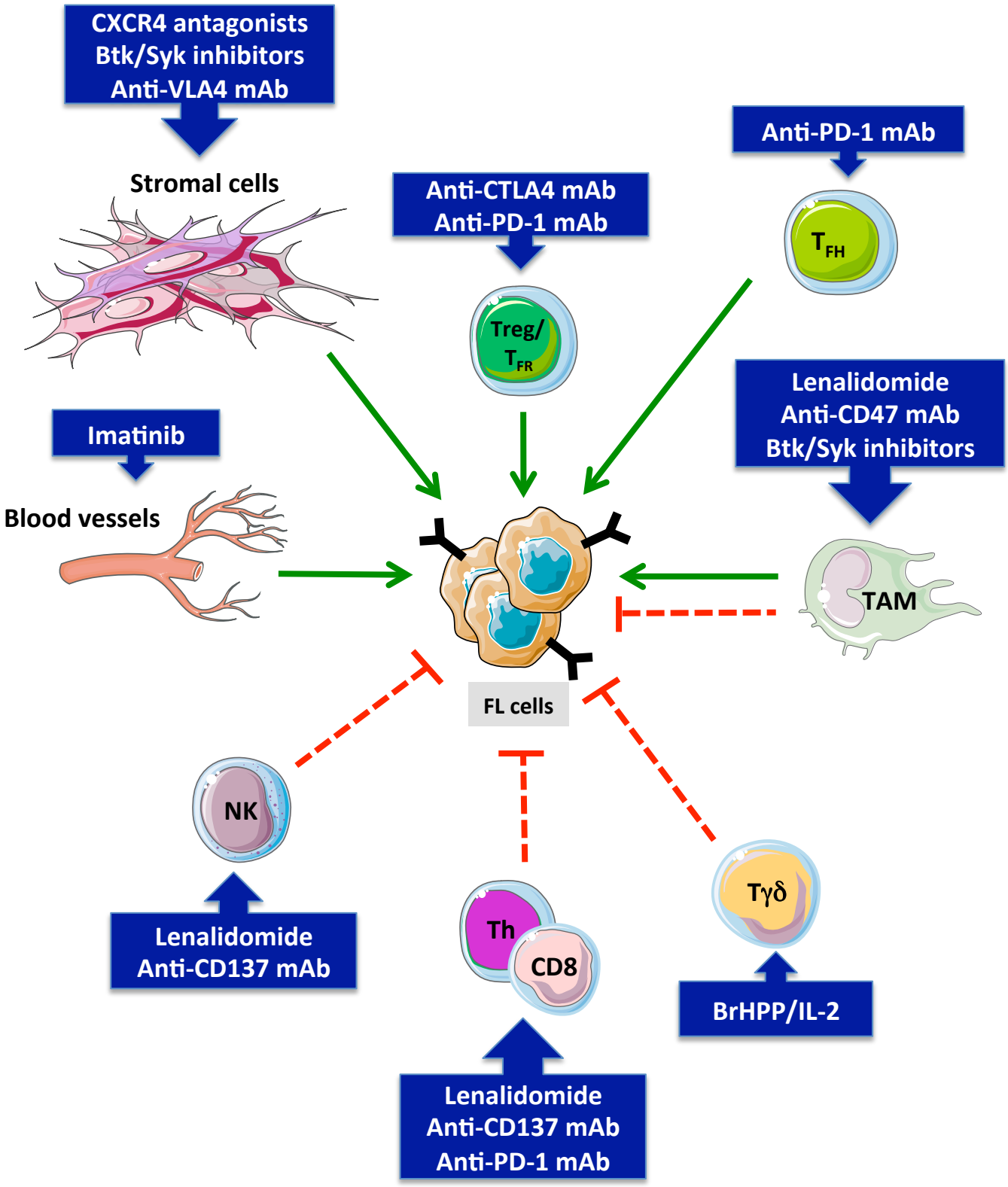
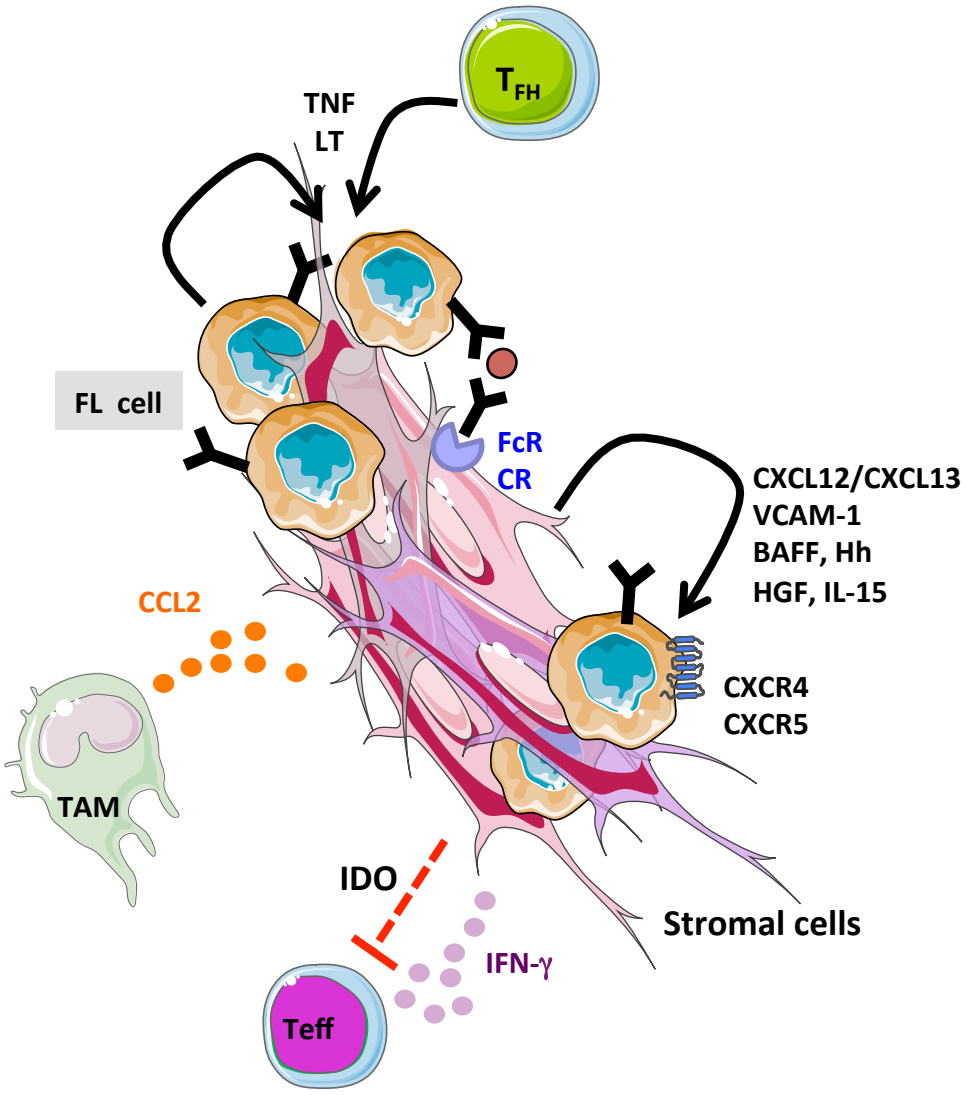

CXCR4 antagonists
Btk/Syk inhibitors
Anti-VLA4 mAb
Stromal cells
Anti-CTLA4 mAb
Anti-PD-1 mAb
Treg/
T FR
Anti-PD-1 mAb
T FH
Lenalidomide
Anti-CD47 mAb
Btk/Syk inhibitors
Imatinib
Blood vessels
TAM
FL cells
NK
Lenalidomide
Anti-CD137 mAb
Th
CD8
Lenalidomide
Anti-CD137 mAb
Anti-PD-1 mAb
Tγδ
BrHPP/IL-2

Figure 4


T FH
TNF
LT
FL cell
FcR
CR
CXCL12/CXCL13
VCAM-1
BAFF, Hh
HGF, IL-15
CCL2
CXCR4
CXCR5
TAM
IDO
Stromal cells
IFN-γ
Teff